

CLAIMS

That which is claimed is:

Sub B5
1. An immunogenic composition, comprising:
a pharmaceutically acceptable excipient; and
live bacteria with DNA adenine methylase (Dam) activity altered relative to wild-type activity of an unaltered pathogenic bacteria, with the alteration being in a manner which renders the bacteria attenuated; and
a first heterologous nucleotide sequence operatively inserted in the bacteria which first heterologous sequence expresses a heterologous antigen.

Sub C
2. The immunogenic composition of claim 1, wherein the Dam activity is altered by an artificially engineered change in the pathogenic bacteria's genome.

Sub D
3. The immunogenic composition of claim 1, wherein the Dam activity is altered by a second heterologous nucleotide sequence.

4. The immunogenic composition of claim 1, wherein the first heterologous sequence is operatively inserted into a first expression cassette.

5. The immunogenic composition of claim 3, wherein the second heterologous sequence is operatively inserted into a second expression cassette.

6. The immunogenic composition of claim 5, wherein the first heterologous sequence is operatively inserted into the second expression cassette.

Sub E
7. The immunogenic composition of claim 1, wherein the genetically engineered change is a non-lethal, non-reverting mutation which renders the bacteria attenuated.

sub
C1
8. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a pathogenic virus.

9. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a pathogenic bacteria.

10. The immunogenic composition of claim 1, wherein the heterologous antigen is a mammalian tumor antigen.

sub
C1
11. The immunogenic composition of claim 1, wherein the heterologous antigen is a mammalian immune disease antigen.

12. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes an enteric infection.

sub
C1
13. The immunogenic composition of claim 12, wherein the microorganism is a bacteria selected from the group consisting of *Enterotoxigenic E. coli*, *Helicobacter pylori*, *Neisseria meningitis*, *Salmonella* (non typhoidal), *Salmonella typhi*, Shiga toxin producing *E. coli*, *Shigella spp.*, and *Vibrio cholera*.

14. The immunogenic composition of claim 12, wherein the microorganism is a virus selected from the group consisting of *Astrovirus*, *Campylobacter*, *Coxsackievirus*, *Echovirus*, *Norwalk virus*, *Poliovirus*, and *Rotavirus*.

sub
C1
15. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes a respiratory infection.

16. The immunogenic composition of claim 15, wherein the microorganism is a bacteria selected from the group consisting of *Influenza virus*, *Measles virus*, *Parainfluenza virus*, *Paramyxovirus*, *Respiratory syncytial virus*, *Rhinovirus*, and *Rubella virus*.

17. The immunogenic composition of claim 15, wherein the microorganism is a bacteria selected from the group consisting of *Bordetella pertussis*, *Chlamydia pneumoniae*, *Haemophilus influenzae B*, NT *Haemophilus influenzae*, *Moraxella catarrhalis*, *Mycobacterium tuberculosis*, *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa*, Smallpox, *Staphylococcus aureus*, Streptococci, Group A (GAS), Streptococci, Group B (GBS) and Tetanus.

SUB 18. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes a sexual transmitted disease.

19. The immunogenic composition of claim 18, wherein the microorganism is a bacteria selected from the group consisting of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Treponema pallidum*.

SUB 20. The immunogenic composition of claim 18, wherein the microorganism is selected from the group consisting, of HIV and Human Papillomavirus.

21. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes a herpes virus infection selected from the group consisting of Cytomegalovirus, Epstein-Barr virus, Herpes simplex II, Herpes simplex II and Varicella zoster virus.

SUB 22. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes a hepatitis virus infection selected from the group consisting of Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E and Hepatitis G.

23. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism selected from the group consisting of *Leptospira spp.*, *Staphylococcus saprophyticus* and Uropathogenic *E. coli*.

24. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes a fungal infection.

25. The immunogenic composition of claim 24, wherein the microorganism is a fungi selected from the group consisting of *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Candida spp.*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum* and *Paracoccidioides brasiliensis*.

26. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes a parasitic infection.

27. The immunogenic composition of claim 26, wherein the microorganism is selected from the group consisting of *Ascaris lumbricoides*, *Entamoeba histolytica*, *Enterobius vermicularis*, *Giardia lamblia*, *Mycobacterium leprae*, *Plasmodium spp.*, *Schistosoma spp.*, *Taenia*, *Toxoplasma gondii* and *Trichomoniasis vaginalis*.

28. The immunogenic composition of claim 1, wherein the heterologous antigen is an antigen of a microorganism which causes a vector borne infection.

29. The immunogenic composition of claim 28, wherein the microorganism is selected from the group consisting of Arbovirus, *Bacillus anthracis*, *Borrelia burgdorferi*, Dengue viruses, Japanese encephalitis virus and Rabies virus.

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